The C-Terminal Region of the α Subunit of Escherichia coli RNA Polymerase Is Required for Transcriptional Activation of the Flagellar Level II Operons by the FlhD/FlhC Complex

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A number of transcription activators have been found to activate transcription via protein-protein contact between RNA polymerase α subunits and transcription factors; they are classified as class I factors. In this report, we demonstrate that the FlhD/FlhC complex, a transcription activator of the Escherichia coli flagellar regulon, requires the C-terminal domain of the RNA polymerase α subunit for transcription activation. We conclude that FlhD/FlhC is a class I transcription factor.

The transcription of Escherichia coli flagellar operons is ordered in a hierarchy, allowing coordinated expression (10, 11). The apex of the hierarchy is occupied by the flhD operon (level I), which expresses two genes, flhD and flhC. Level II consists of genes encoding many structural components assembled in the early and middle stages of flagellar synthesis, as well as a flagellum-specific sigma factor (σ28). Level III operons include genes for late morphogenesis, motor rotation, and chemotactic signaling (9, 15, 16).

The E. coli RNA polymerase (RNAP) holoenzyme is a protein composed of five subunits, αββ′α′. The sigma factor subunit determines the promoter specificity of RNAP, whereas the core enzyme (αββ′) is the basic machinery for RNA synthesis. The alpha subunit plays a critical role in subunit assembly and transcriptional activation. It consists of two independently folded domains (N- and C-terminal) with an interdomain linker (1, 18). The N-terminal region is involved in subunit-subunit contacts for core enzyme assembly (3, 4), whereas the C-terminal domain is required for interactions with some transcription activators (5–7). The C-terminal region is also capable of dimerization (1) and DNA binding (19). These transcription activators cannot activate transcription with mutant RNAPs containing C terminally truncated α subunits and are termed class I factors (8). Other transcription factors, including class II factors, can be distinguished from class I factors by the fact that they do not interact with the C-terminal domain of the α subunit. The requirement for the α subunit suggests that class I factors interact with the C terminus of the α subunit. Most of the class I factors bind to target DNA at sites upstream from the −35 region of promoters (8).

Genetic analysis of flagellar gene expression in E. coli suggests that FlhD and FlhC are the master regulatory proteins of the flagellar transcriptional regulator (10). Biochemical studies show that these two proteins form a complex. This complex binds to the upstream control regions of flagellar level II genes and activates transcription of those level II operons (14). Although it is known that the FlhD/FlhC complex is a transcriptional activator of the E. coli flagellar level II operons, little is known about the mechanism by which FlhD/FlhC influences transcription initiation by RNAP. On the basis of DNase I footprint data, it has been suggested that transcriptional activation of flagellar level II operons by the FlhD/FlhC complex involves direct contact between the bound FlhD/FlhC and RNAP (14). In this report, we take advantage of two RNAP mutants, each with a deletion in the C-terminal region of the α subunit, to address the question of whether activation by FlhD/FlhC indeed involves specific interaction between FlhD/FlhC and RNAP.

The mutant RNAPs containing C terminally truncated α subunits used in this study were prepared as described by Igarashi and Ishihama (6). Core RNAPs were reconstituted from isolated β, β′, and wild-type or C terminally truncated α subunits. The holoenzymes were reconstituted by mixing reconstituted core enzymes and a fourfold molar excess of the σ70 subunit. The specific activities of poly(dA-dT)-dependent poly(A·U) synthesis were essentially the same for the three reconstituted core enzymes. However, the specific activities of promoter-dependent transcription varied, depending on the promoter, for the three reconstituted holoenzymes. When DNA fragments containing the lacUV5 promoter were used as the templates for in vitro transcription, the specific activities for the wild-type, mutant α-256, and mutant α-235 enzymes were 4:1:1 (data not shown).

We examined the ability of purified FlhD/FlhC to activate the initiation of transcription in vitro by these reconstituted holoenzymes. All three level II promoters, fliA, flhB, and fliL, used as transcription templates for FlhD/FlhC were PCR products prepared as previously described (14). As shown in Fig. 1, the 248-bp DNA fragment which extends from −147 to +100 was used to measure transcription from the fliA promoter, transcription from the 334-bp fragment for the flhB promoter gave a 201-nucleotide transcript, and the 358-bp fragment of the fliL promoter gave a 126-nucleotide transcript. A 550-bp fragment containing the lacUV5 promoter was gel purified from HindIII and EcoRI digestions of pJM6 (17) and included in reaction mixtures as an internal control. FlhD/FlhC protects a 48-bp region of the fliA operon between positions −41 and −88, a 50-bp region of the flhB operon between positions −28 and −77, and a 48-bp region of the fliL operon between positions −29 and −76. The binding center of FlhD/
FlhC is located at -64 for fliA and -52 and -55 (top and bottom strands, respectively) for both flhB and fliL.

Purified FlhD/FlhC proteins can activate transcription in vitro from the fliA, flhB, and fliL promoters by wild-type RNAP holoenzyme containing s70 (14). To examine whether the C-terminal domain of the RNAP α subunit is necessary for transcriptional activation of flagellar level II operons by the FlhD/FlhC complex, we carried out in vitro mixed transcription with two mutant RNAPs. Mixtures of the lacUV5 promoter and one of the three level II promoters, fliA, flhB, or fliL, were preincubated at 37°C for 20 min in the presence or absence of FlhD/FlhC (0.5 μg), after which reconstituted RNAP holoenzyme (0.5 pmol of the wild type or 2 pmol of a mutant holoenzyme) was added and incubated for additional 15 min to allow open complex formation. Then a mixture of nucleoside triphosphates, [α-32P]UTP, and heparin was added, and transcription was allowed to proceed for 15 min at 37°C. The reactions were stopped by extraction once with phenol-chloroform (1:1) and then once with chloroform. The samples were denatured at 70°C for 5 min and subjected to electrophoresis on 8 M urea–acylamide gels. The gels were analyzed with an Ambis β-scanning and imaging system prior to exposure to Kodak XA-R film.

As shown in Fig. 2, in the absence of FlhD/FlhC, no transcripts were synthesized from the fliA (A), flhB (B), and fliL (C) promoters for wild-type and mutant RNAPs (lanes 1, 3, and 5). When FlhD/FlhC was added, transcripts from those three promoters were observed for the wild-type enzyme (Fig. 2, lanes 2), but not for the two mutant holoenzymes (lanes 4 and 6). The results obtained for the reconstituted wild-type RNAP were consistent with those of previous experiments with native RNAP (14). FlhD/FlhC did not affect transcription from the lacUV5 promoter with either wild-type or mutant RNAPs, indicating that the FlhD/FlhC complex acts specifically on transcription from flagellar promoters. This result clearly demonstrates the involvement of the α subunit C-terminal region in transcription activation by FlhD/FlhC. This finding supports the theory that direct protein-protein contact between the FlhD/FlhC complex and the α subunit of RNAP is involved in transcription initiation. We consider the FlhD/FlhC complex to be a class I transcription factor, even though the binding sites of FlhD/FlhC overlap the promoter −35 region in flhB and fliL or are near −40 in the case of fliA. The same phenomenon has been observed for the following class I transcription factors: Ada (20), OxyR (21), TrpI (2), and TyrR (12) proteins, which bind to DNA sites partially overlapping the −35 signal. In conclusion, the FlhD/FlhC complex is a class I transcription activator which requires the C-terminal domain of the RNAP α subunit.

A. α-253  - - - - +  
 α-256  - - - - -  
 WT    + + - - -  
 D/C   - + - + -  
 PfliA 1 2 3 4 5 6  

B. α-253  - - - - +  
 α-256  - - - - -  
 WT    + + - - -  
 D/C   - + - + -  
 PfhlB 1 2 3 4 5 6  

C. α-253  - - - - +  
 α-256  - - - - -  
 WT    + + - - -  
 D/C   - + - + -  
 PfliL 1 2 3 4 5 6  

FIG. 2. In vitro mixed transcription by wild-type (WT) and mutant RNAPs containing C terminally truncated α subunits (α-256 and α-235). The composition of each reaction mixture is given above the corresponding lane. + or −, presence or absence, respectively; D/C, the FlhD/FlhC complex. Arrows indicate the specific transcripts initiated at the corresponding promoters. PflA, PfhlB, and PflL (A), fliA (B), and fliL (C) promoters, respectively.
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